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AXIAL SUSCEPTIBILITY GRADIENTS IN THE EARLY DEVELOPMENT OF THE SEA URCHIN.

C. M. CHILD.

(WITH 20 FIGURES.)

Axial gradients in susceptibility to cyanides and various other agents have already been demonstrated in *Planaria* (Child, '13*b*) various infusoria (Child, '14), the early developmental stages of the starfish (Child, '15*a*), in a number of species of Oligochetes (Hyman, '16), in a number of algæ and in various other animals the data for which are as yet unpublished. Thus far such gradients have been found, at least in the earlier stages of development in all forms examined, comprising more than sixty species and including algæ, cœlenterates, flatworms, echinoderms, annelids, and vertebrates. The relation of these gradients to developmental gradients of other kinds and the problem of their significance for the physiological individual has also been considered (Child, '15*b*, Pt. III, '15*c*).

During the summers of 1913 and 1915 at the Marine Biological Laboratory, Woods Hole, Mass., axial susceptibility gradients were demonstrated in the early developmental stages of the sea urchin, *Arbacia punctulata*, and the control and modification of development by means of the differential susceptibility along these gradients was found to be possible. In the present paper only the direct evidence for the existence of such gradients, based on the progress of death and disintegration along the axis, is considered and this is incomplete in certain respects. The indirect evidence from the control and modification of development, with which I was chiefly concerned and which is of greater interest, will be presented in another paper.

METHODS OF DEMONSTRATING SUSCEPTIBILITY GRADIENTS.

The method employed in demonstrating the gradients has already been described (Child, '13*a*, '15*b*, Chap. III.) and consists in directly determining the differences in susceptibility along

the axis or axes to cyanides and various other agents used in concentrations sufficient to kill in the course of a few hours, but not high enough to kill immediately and not low enough to permit the organisms to become acclimated or acquire a tolerance to them. The agents used in these studies on the sea urchin were potassium cyanide, ammonium hydrate, ethyl alcohol and hydrochloric acid in sea water. The various developmental stages were placed in concentrations of these substances determined by preliminary experiment and the progress of death along the axis was observed.

In many of the lower animals the progress of swelling, cytolysis, separation and disintegration serves directly as an indication of the progress of death. In the developmental stages of the sea urchin changes of this sort occur as the cells die, but they differ somewhat with different reagents and different stages of development. In KCN the cells swell, become spherical, and separate from each other as they die and the region concerned breaks down into a shapeless mass of these spherical cells which soon disintegrate. If motor activity is still present in other parts the dead cells may be progressively left behind as the living portion moves about. This disintegration of the body is more marked in the earlier stages of the blastula and gastrula than in later stages where supporting tissues have differentiated, but even in the later stages extensive disintegration can be brought about by return to sea water after a sufficient length of time in KCN. These death changes are somewhat accelerated and intensified by the return to water and death is marked by very complete disintegration. Thus when KCN is used, the progress of death can either be followed directly under the microscope in the KCN solution, at least in the blastula and gastrula stages, or lots may be returned to water at stated intervals and the progress of death determined by the comparison of dead and living portions of the body in successive lots. Both methods have been used, but the latter is more satisfactory in many cases, because after return to water the dead portions disintegrate rapidly and completely while the parts which are still alive may recover and resume motor activity where the stages in which movement occurs are concerned. The progress of death can also be made

visible by staining with neutral red before placing in KCN. When death occurs the neutral red color changes to yellow, as the alkali of the KCN solution penetrates the cells, and then disappears.

The death changes in ammonium hydrate are very similar to those in KCN. The cells swell and in the blastula and gastrula stages separate, but in later stages the cells cohere more or less after death and the visible death changes are merely swelling and rounding of the cells and a consequent increase in size and greater translucency of the whole. Here likewise, return to water increases the disintegration and so makes it easier to follow the progress of death, and neutral red may also be used as an indicator of death in the same way as with KCN.

Ethyl alcohol is used in the same way as KCN and NH_4OH , but of course in much higher concentration. Disintegration of blastula and gastrula stages is very complete and in the plutei only the supporting tissues retain the body form.

In hydrochloric acid, however, the behavior of the cells is different, as might be expected. The cells shrink and do not separate and death may occur with very little visible change except in size. But return to sea water after a sufficient length of time in HCl brings about disintegration and the dead and dying cells swell and separate so that the progress of death can be followed without difficulty by removing lots from HCl to sea water at regular intervals.

As regards concentration of the reagents used, a wide range is possible according to the stage of development and the length of survival time desired. Since the susceptibility increases very greatly from fertilization up to the blastula stage as physiological rejuvenescence occurs (Child, '15b, pp. 412-418), much higher concentrations can be used for the former than for the latter stages. At a temperature of $22-24^\circ \text{C}$. unfertilized eggs begin to die after 4-5 hours in KCN $m/100$, while blastulæ and later stages begin to die after the same length of time in KCN $m/1,000$. The other reagents were used only on the blastula and later stages. In NH_4OH $m/500$ death of these stages begins in half an hour to an hour, in $m/1,000$ in 1-2 hours. The same stages in alcohol 4 per cent. (roughly $m\ 2/3$) begin to die in 3-4

hours and after 5-10 minutes in HCl $m/400$ death begins on return to water, but it is difficult to determine just when it occurs, if the stages are left in HCl. Much lower concentrations of KCN and NH_4OH can be used without the occurrence of any appreciable degree of acclimation, but acclimation to alcohol and HCl takes place much more rapidly and in relatively high concentrations, alcohol 1.5 per cent., HCl $m/2,000$, so that for direct demonstration of the susceptibility gradient with these reagents the range of concentration is not so great.

The significance of differences in susceptibility to cyanides, various narcotics and certain other agents has been considered elsewhere (Child, '13a, '15b, Chap. III.). It has been found that in concentrations high enough to kill without acclimation the susceptibility varies in general directly with the rate of metabolism or of certain fundamental reactions, while in concentrations low enough to permit acclimation the higher the metabolic rate the greater the degree and rapidity of acclimation consequently in the long run the susceptibility to these concentrations varies inversely as the metabolic rate.

Although the susceptibility method has proved in certain cases to be a very satisfactory means of distinguishing differences in general metabolic activity, it is actually of course a rather crude method and merely makes it possible to compare in a rough way metabolic differences in different individuals or body regions and does not of course give us any exact quantitative data. Moreover, the nature of the method makes it evident that we cannot expect to distinguish with certainty the minuter metabolic differences, because the reagents used decrease to some extent the differences which they are expected to show. Definite and constant differences in susceptibility must mean considerable differences in rate of reaction, but the absence of such differences in susceptibility does not necessarily mean the complete absence of differences in rate of reaction. Notwithstanding these limitations, however, the method is useful and the positive results obtained by means of it afford ample proof of its value.

One further point requires some consideration. The method has been criticized, more particularly in personal conversation, as involving certain assumptions concerning the action on living

chemically active protoplasm of cyanides and other agents used. This, however, is not the case. There is no reason for believing that different agents which retard or inhibit metabolic activity in living protoplasm all act in the same way. Protoplasm is a complex system in which both the physical substratum and the chemical reactions play a part, but the important point is that it is a system, *i. e.*, that the different processes, changes and conditions in it are not independent of each other but mutually correlated and dependent to a greater or less degree. It is not in the least improbable that different agents may give the same general results, as regards susceptibility, even though one acts primarily on the aggregate condition of the colloids or let us say the permeability of membranes, another on the production or constitution of enzymes, and still another on the chemical reactions of oxidation. My observations on susceptibility to cyanides, various narcotics, acids, alkalies, metabolic products, and even temperature have convinced me that the relation between susceptibility to retarding or inhibiting agents and conditions and general metabolic rate or the rate of certain fundamental metabolic reactions is a very general relation and there seems to be absolutely no reason for believing that it is dependent upon any one particular method of action on the protoplasmic system of the agent or condition employed. It still remains of course for future investigation to determine the exact method of action of each agent and condition, to formulate the general rule and to discover and account for exceptions if they exist. Since our present knowledge indicates both that the oxidations are fundamental metabolic reactions and that they are more or less dependent on various conditions in the protoplasmic system; we may expect to find that their rate is altered by a great variety of external agents and conditions even though these do not enter directly into the chemical reactions of oxidation.

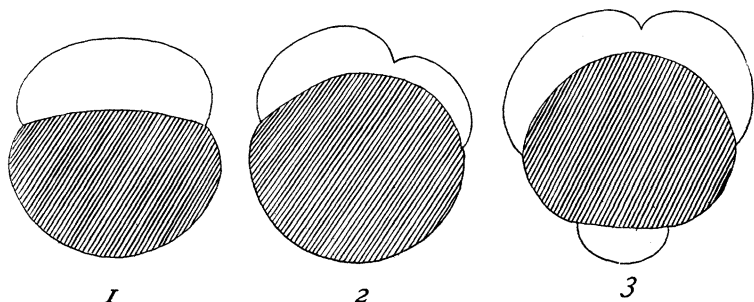
In certain cases, as in the green plants, the energy for certain of the synthetic or anabolic reactions is derived directly from sources outside the organism and in such cases these reactions may be to a considerable degree independent of the energy-producing reactions in the organism. Such reactions, however,

are in a sense only preliminary, although of course essential to the fundamental processes of life and the relation between susceptibility and metabolic rate is primarily concerned, not with them, but rather with the reactions which play a fundamental rôle in setting free the energy characteristic of living organisms.

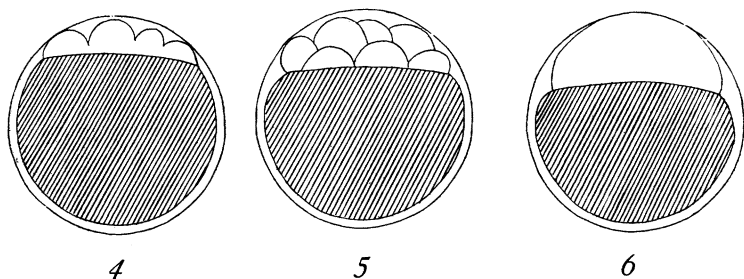
In the sea urchin the differences in susceptibility along the axis so far as observed, are essentially the same whether cyanides, alcohol, alkalies or acids are used as reagents. All these substances interfere with the action of the protoplasmic system in some way, but by no means necessarily in the same way, yet the general results as regards susceptibility are the same. This fact is highly significant and indicates to some extent the general character of the relation between susceptibility and the fundamental conditions and processes of life.

THE EGGS AND CLEAVAGE STAGES.

Since experiments on the control and modification of development through differential susceptibility along the axes had shown very conclusively the existence of axial gradients in the early developmental stages and had indicated their probable presence even in the unfertilized egg, and since it was soon found that the character of the death changes made it difficult to reach definite conclusions concerning death gradients from direct observation, but little time was spent on these earlier stages and the evidence obtained, so far as it has any value, is merely contributory. I regret to state, however, that I neglected to employ as a means of orientation the method of making the micropyle visible by colored suspensions in the water. This might have made the data on these earlier stages somewhat more conclusive.



The unfertilized eggs in KCN $m/100$ show death changes of the character indicated in Figs. 1-3. First, one or more clear droplets or masses which contain some colloids and much water appear on the surface of the egg and grow larger, while the granular portion decreases in size. This process may go on, the clear masses often uniting, until the clear and granular portions are almost equal in size, but finally the granules spread and the

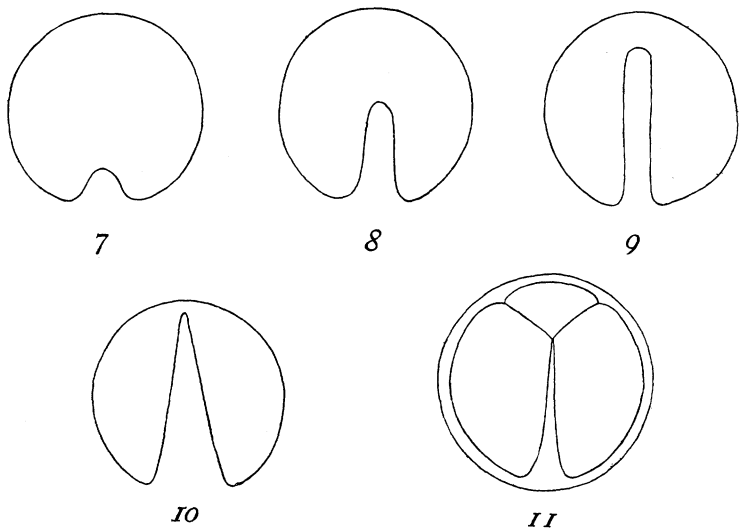


distinction between clear and granular areas disappears. The only indication of a susceptibility gradient is the appearance of the clear droplets only or earlier on one hemisphere or at one pole. Within certain limits of concentration this has been observed in some 70-80 per cent. of the eggs, but in some eggs the clear droplets appear at opposite poles or over various parts of the surface at the same time. The fertilized eggs before cleavage (Figs. 4-6) behave in KCN essentially like the unfertilized.

The blastomeres of the earlier cleavages show a similar separation into clear and granular areas and here again the clear areas or droplets usually appear first or only on one hemisphere or at one pole of the egg. In eggs placed in KCN after the appearance of the micromeres I believe that the apical hemisphere is in general more susceptible than the basal.

An interesting gradient in the first cleavage appears in certain concentrations of cyanide. In such cases the first cleavage plane at its first appearance extends as a furrow only half way or less around the egg and cuts through the egg in one direction without the appearance of any furrow on the opposite side (Figs. 7-10). In some cases a larger or smaller portion of the cytoplasm is separated from the two blastomeres at the end of this cleavage

by the division of the cleavage furrow into two (Fig. 11). Such cleavages occurred in about 40 per cent. of eggs placed in KCN $m/1,000$ for $10\frac{1}{2}$ hours before fertilization then well washed and kept in sea water and fertilized one hour after removal from KCN. Figs. 7-10 are drawn from such eggs. After this treatment there



is no elevation of a fertilization membrane from the egg surface, and the blastomeres frequently become entirely separated after cleavage. Eggs placed in KCN $m/100$ fifteen minutes after fertilization and washed and returned to sea water after three hours also showed cleavage of this kind in 30-40 per cent. In the other eggs of these lots cleavage is normal or incomplete and often irregular or else delayed with simultaneous formation of a number of blastomeres. Apparently then these one-sided cleavages represent the first step in departure from normal cleavage. The indirect evidence shows that a susceptibility gradient is present in these stages of development and that the region of highest susceptibility and therefore of highest metabolic rate is the apical pole. It is evident that cyanide inhibits cleavage, and if the apical pole is most susceptible, we should expect to find cleavage most completely inhibited there, and least inhibited at the basal pole. It is probable, therefore, that these cleavage furrows start from the basal and proceed toward the apical pole,

and that in cases like that in Fig. 11, where a portion of the cytoplasm is cut off, it is the apical region, which is so much injured that it cannot give rise to a cleavage furrow.

With the same methods of treatment a general gradation in the size of the blastomeres was observed in 30-40 per cent. of the eggs in stages from 32 cells onward, though it became less marked in later stages as recovery proceeded. In these cases also it is probable that the region of most rapid cleavage and therefore of the smallest blastomeres is the basal region and the region where cleavage is most inhibited and therefore the blastomeres are largest is the apical region.

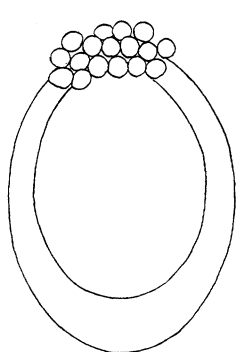
The small size and protoplasmic character of the micromeres at the basal pole suggests the possibility that their metabolic rate may be higher than that of the cells about them, but I have not been able to discover that their susceptibility is greater than that of the adjoining cells, and the indirect evidence shows that the mesenchyme cells, which are believed to be at least in part descendants of the micromeres are among the least susceptible if not the least susceptible of all the cells.

These observations on the earlier developmental stages are not conclusive. Taken by themselves they are of little value, but considered in the light of the indirect evidence and of the more definite results obtained in later stages they possess a certain significance as contributory evidence. My observations on these stages were only incidental to other work and only KCN was used as reagent.

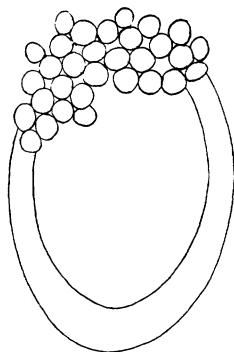
THE BLASTULA AND GASTRULA.

As soon as the blastula begins to elongate the direction of movement, and in more advanced stages the greater thickness of the cellular wall in the basal region make it possible to distinguish apical and basal ends without difficulty. In these stages death begins at the apical end and proceeds basally (Figs. 12-15), advancing in many cases somewhat more rapidly down one side (Figs. 13, 14), probably that side which later becomes the anterior end of the pluteus. The progress of death in the basal direction is very regular, though a few cells here and there may swell and push out of the body-wall earlier than others

about them. The susceptibility gradient is the same with all the reagents and methods of use noted in the preceding section so that there can be no doubt of the existence of an apico-basal gradient which is fundamentally related to the activity of the

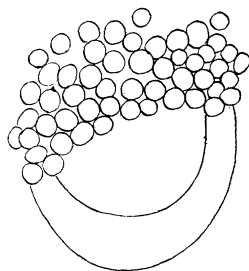


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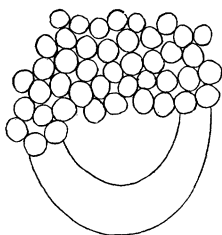


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living protoplasm. The more rapid progress of death down one side of the blastula which is frequently observed is probably an indication of differences in other axes, but on this point certainty is impossible.



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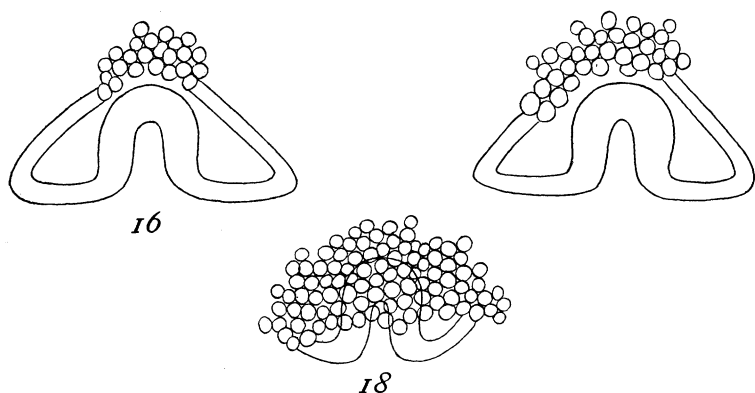


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In the gastrula stage the same gradient appears (Figs. 16-18), the apex of the gastrula, which represents the apical region of the egg and blastula, being most susceptible, the basal least susceptible. The susceptibility of the entoderm and the blastopore region is very much lower than that of the other ectodermal regions. The entoderm is still intact after practically the whole ectoderm has disintegrated (Fig. 18) and in concentrations where

ectodermal disintegration occurs in three or four hours disintegration of the entoderm is usually not complete until one or two hours later or in some cases even a longer time.

By returning to sea water after the proper length of time in the

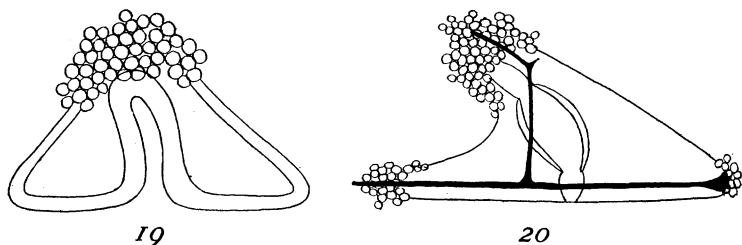


reagent it is possible to stop death at any level of the body and to bring about recovery and further development of the parts still alive. In the blastula it is more or less of the basal portion which remains alive, and this may close up and gastrulate, producing dwarf gastrulæ with a disproportionately large enteron. In the gastrula stage also partial dwarf gastrulæ with large enteron result from partial disintegration and recovery, because the more apical ectoderm may be in large part destroyed while the basal region of the gastrula and the entoderm remain intact. The forms of larvæ which develop from these partial basal blastulæ and gastrulæ will be described in another paper.

LATER STAGES.

In the early stages of transformation into the pluteus, the gastrula loses its apparent radial symmetry and becomes triangular in outline in basal or anal view, the base of the triangle representing the anterior region of the future pluteus. In side view the apex of the gastrula is seen to be shifted toward the anterior end as compared with earlier stages (*cf.* Figs. 19 and 16). In the further transformation this apical region becomes the oral lobe of the pluteus and the long anal arms develop from the basal region of the anterior end (Fig. 20).

The susceptibility gradient in the earlier stages of this transformation is the same as in the gastrula, apico-basal (Fig. 19) and the same difference in susceptibility between ectoderm and entoderm persists. Later, when the larva begins to elongate in



the antero-posterior direction, and the anal arms begin to develop, these arms and the posterior end both appear as secondary regions of high susceptibility (Fig. 20) though the susceptibility of the anal arms is in general somewhat less than that of the oral lobe and that of the posterior end somewhat less than that of the anal arms. On the oral lobe and over the body death progresses in the basal and posterior direction and in the anal arms from tip to base of the arms.

In the fully developed pluteus the susceptibility gradients are less marked. The ectoderm of the oral lobe and anal arms is still somewhat more susceptible than that of other regions but the differences are less conspicuous. In all these later stages, however, the entoderm remains much less susceptible than the ectoderm and apparently the mesenchyme is least susceptible of all parts.

It is probable that the gradual fading out of the metabolic gradients in the pluteus is a physiological change which precedes and makes possible the development of the axial gradients of the mature sea urchin which have been previously inhibited by the existing axial relations. If this suggestion is correct these changes are the factors which determine, or rather permit metamorphosis.

The development of the arms and the posterior elongation has been shown to be dependent on the development of the skeleton. That being the case the appearance of high susceptibility in the anal arms and the posterior end is not self-determined in these parts but results from skeletal growth.

DISCUSSION AND SUMMARY.

The existence in the apico-basal axis of the blastula, gastrula and later stages of a definite and conspicuous gradient in susceptibility which is the same for cyanide, alcohol, acid and alkali is a significant fact, particularly in the light of the relations between susceptibility to cyanides and various narcotics and general metabolic rate. The results with HCl and NH_4OH show that this relation is the same for these substances. The further evidence for the existence of this gradient in the earlier developmental stages, together with the difficulty of conceiving how an apico-basal gradient could arise *de novo* during the earlier development, leave little doubt that this axial gradient persists from the unfertilized egg to the mature pluteus. This conclusion will be confirmed by the indirect evidence presented elsewhere.

As regards gradients in other axes the evidence is less conclusive. It is probable that the asymmetry in the progress of death frequently observed in the blastula (Figs. 13, 14) is an indication of a difference between anterior and posterior regions and this probability is strengthened by the appearance of a similar asymmetry in the gastrula and prepluteus stages (Figs. 17 and 19).

It must be remembered, however, that this method of determining susceptibility is far from being a perfect method for the demonstration of differences in metabolic rate. It can be expected to show only the grosser differences, for the reagents used tend to decrease the differences which they are used to demonstrate, and if the differences are originally slight they may disappear so rapidly in the reagent that no appreciable or constant differences in the time of death appear.

It is evident from the course of development that the apico-basal gradient is the most strongly marked and in the early stages even this is not very clearly defined by differences in susceptibility as determined by time of death, though it becomes more distinct later. It is not to be expected that the minor differences along the axes of symmetry should appear as clearly by this method as the differences along the major axis. The important facts are that the major axis appears so distinctly as

a susceptibility gradient and that indications of susceptibility differences in the minor axes have been observed.

The apico-basal susceptibility gradient is the same as that in the starfish (Child, '15*a*) and in all other forms examined, at least in early developmental stages and in many cases throughout life. That this gradient is of fundamental significance cannot be doubted and I have attempted elsewhere (Child, 15*c*) to present some of the evidence which seems to me to indicate that a physiological axis is fundamentally such a gradient in metabolic rate.

The chief points are summarized as follows:

1. A distinct gradient in susceptibility to potassium cyanide, ethyl alcohol, ammonium hydrate and hydrochloric acid is present along the apico-basal axis of blastula, gastrula, and later stages of larval development of *Arbacia* and indications of a gradient are found in earlier stages.
2. In the apico-basal gradient the susceptibility is highest at the apical end of the axis and lowest at the basal end. Since susceptibility to these reagents varies in general directly with metabolic rate, the susceptibility gradient indicates the existence of a gradient in rate of metabolic activity in which the rate is highest in the apical region and decreases basally.
3. Some indications of gradients in other axes appear in the differences of susceptibility, but these are much less distinct than the differences along the apico-basal axis.
4. The anal arms and the posterior end of the larval body appear as secondary regions of high susceptibility after they begin to develop.
5. In the fully developed pluteus these susceptibility gradients become less marked and doubtless disappear as metamorphosis begins.

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February, 1916.

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